

AD_____

Award Number: DAMD17-00-1-0597

TITLE: A New Perspective on DCIS Using MRI: Correlation of
Tumor and Vessel Proliferation with MR Signal Enhancement

PRINCIPAL INVESTIGATOR: Laura J. Esserman, M.D.
Nola Hylton, M.D.

CONTRACTING ORGANIZATION: University of California
San Francisco, California 94143-0962

REPORT DATE: October 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20030411 026

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE
October 2002

3. REPORT TYPE AND DATES COVERED
Annual (1 Oct 01 - 30 Sep 02)

4. TITLE AND SUBTITLE

A New Perspective on DCIS Using MRI: Correlation of Tumor and Vessel Proliferation with MR Signal Enhancement

5. FUNDING NUMBERS
DAMD17-00-1-0597

6. AUTHOR(S)

Laura J. Esserman, M.D.
Nola Hylton, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California
San Francisco, California 94143-0962

E-Mail: laura.esserman@ucsfmedctr.org

8. PERFORMING ORGANIZATION
REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING
AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The purpose of this study is to correlate density of contrast enhancement on breast MRI images with pathology characteristics and markers of proliferation and angiogenesis in women with ductal carcinoma in situ (DCIS) of the breast. The specific aims of the study are two-fold. 1) We will first develop a novel method for characterizing DCIS lesions based on cellular proliferative activity and angiogenesis within the tumor and surrounding vascular endothelium. Using immunohistochemical techniques, we will determine the relationship between proliferation and angiogenic activity in the range of DCIS detected. 2) Secondly, we will correlate the proliferative and angiogenic profile with MRI characteristics in order to determine whether MR can predict the biological characteristics of DCIS. Thus MR could potentially serve as a surrogate marker of biological behavior. These two aims will lead to a better understanding of the basis and timing for transformation of DCIS which would help us to find more optimal ways to treat DCIS and help us to treat invasive breast cancer and develop strategies for prevention.

14. SUBJECT TERMS

ductal carcinoma in situ (DCIS), MRI, breast cancer

15. NUMBER OF PAGES

13

16. PRICE CODE

17. SECURITY CLASSIFICATION
OF REPORT

Unclassified

18. SECURITY CLASSIFICATION
OF THIS PAGE

Unclassified

19. SECURITY CLASSIFICATION
OF ABSTRACT

Unclassified

20. LIMITATION OF ABSTRACT

Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover	1
SF 298	2
Table of Contents	3
Introduction	4
Body	4
Key Research Accomplishments	6
Reportable Outcomes	6
Conclusions	6
References	8
Appendices	11

Introduction

The purpose of this study is to correlate density of contrast enhancement on breast MRI images with pathology characteristics and markers of proliferation and angiogenesis in women with ductal carcinoma in situ (DCIS) of the breast. The specific aims of the study are two-fold. 1) We will first develop a novel method for characterizing DCIS lesions based on cellular proliferative and angiogenic activity within the tumor and surrounding vascular endothelium. Using immunohistochemical techniques, we will determine whether this proliferation is found in the DCIS itself, in the surrounding stroma, or in vascular endothelial cells and whether they are proximate. 2) Secondly, we will correlate this angiogenic and proliferative profile with MRI characteristics in order to determine whether MR can predict the biological characteristics of DCIS. Thus MR could potentially serve as a surrogate marker of biological behavior. These two aims will lead to a better understanding of the basis and timing for transformation of DCIS which would help us to find more optimal ways to treat DCIS and help us to treat invasive breast cancer and develop strategies for prevention.

Body

Specific Aim 1: Stain a series of 90 DCIS lesions and 20 non-malignant controls with proliferative, epithelial, endothelial and stromal markers.

Accomplishments:

- We have obtained informed consent for patients with a diagnosis of ductal carcinoma in situ (DCIS) who have had an MRI prior to definitive surgery.
- We have obtained a waiver of informed consent to analyze samples and MR images from patients who have undergone a preoperative MRI for a diagnosis of DCIS.
- We have completed review of 20/90 pathological slides and have designated corresponding tissue blocks to be used in our immunohistochemistry analysis. All sections will be stained by mid November.

Task 1.1: Create a database of 75 patients with DCIS which includes details of physical findings and mammographic presentation.

- We have created a database of patients with DCIS that includes detailed physical findings and mammographic presentation.
- We have 90 patients in this database and have complete information on their surgery type, MRI date, surgery date, age at diagnosis, size, type, grade, necrosis, extent, number of segments involved, pixel density, and pattern of enhancement. Because of the change in personnel in radiology, we will not have the MRI analysis complete until mid November.

Task 1.2: Identify 10 non-malignant controls that do not enhance like cancer and 10 malignant lesions that enhance on MR.

- We have 10 control cases that are fibroadenomas.
- We have identified 5 suitable non-malignant lesions that look like cancer.
- We expect to identify 5 more suitable controls by December 2002.

Task 1.3: Review all pathology in terms of grade, extent, size and patterns of tumor vessels by H&E.

- Review of pathology was delayed due to staff turnover, including the lead pathologist, Dr. Dan Sudilovsky, the breast surgery fellow involved on the study, Dr. Veronica Shim, the study coordinator and a large increase in radiology clinical volume which decreased the time available for this study by Dr. Wolverton. Staff involved on the study has stabilized and we expect to maintain consistent progress on the study. We are expecting a new full-time radiologist to start at UCSF on November 1, which will free time to complete the study.
- We are completing review of pathology slides under the current lead pathologist, Dr. Yunni Chen, and expect to complete pathological review by December 2002.

Task 1.4: Stain tumor specimens using CD 34 and CD 105 in order to highlight vascularity of tumor lesions.

- A comparison of CD 34 and CD 31 has been performed and CD 31 has been chosen due to its comparable degree of sensitivity and the fact that it produces less background noise.
- CD 105 has been chosen as a stain as it is expressed in vessels characteristic of tumors.
- It has been determined that the cut section will be better than the core for quantitatively counting microvessels to assess angiogenesis.
- We anticipate completion of staining by December 2002.

Task 1.5: Add serial section stain and dual stain with proliferative markers to elucidate which areas of tumor are proliferating.

- We will add serial section stains rather than dual stains with preoliferative markers (Ki67, cytokeratin, and MCM2) to elucidate whether tumor, epithelial, or endothelial areas are proliferating. The double staining methods add confusion rather than clarity.
- If upon completing review of first 30 slides, the results of both Ki67 and MCM2 stains are the same, we will continue with the the Ki67 stain and eliminate the MCM2 stain.

Task 1.6: Compare proliferative patterns of tumor and blood vessels and correlate to grade, extent, and Her2/neu markers.

- We are in the process of assessing Her2 and will compare this to grade and extent determined from initial pathological slide review.

Task 1.7: Identify stromal marker to elucidate involvement of stromal cells in tumor lesions.

- We are in the process of reviewing stromal stains and identifying the appropriate stain to be used in this study. Candidate stains include CD44 and SMA 1 & 2.

Specific Aim 2: Create a tissue array from DCIS cases to see if this technique can be used to capture the same data described in Specific Aim 1.

Task 2.1: Create tissue array composed of plugs from identified tissue blocks.

- A tissue array from the DCIS cases is being created by Dr. Karen Chew as the pathology slide review progresses.

Task 2.2: Stain tissue sections as in Specific Aim 1 and correlate individual sections with tissue arrays.

- This process is also ongoing as pathology slide review is completed.

Specific Aim 3: Define MRI Characteristics of DCIS

Task 3.1: Examine and compare all MR images; create a stratification and standards of patterns seen based on extent, density, and intensity of contrast enhancement of study cases.

- We are currently testing our method for identifying the pixels to measure MRI density.
- All MR images will be analyze/characterized according to morphological changes. Our new radiologist, Dr. Jessica Leung, will complete this review by December 2002.

Task 3.2: Categorize each image according to these patterns of extent, density, SER, and imaging phenotype.

- Dr. Nola Hylton has defined a set standards in classifying MR images according to these patterns.
- These standards will be applied to the categorization of all case MR images by January 2003.

Specific Aim 4: Investigate associations between MR, proliferative markers, and standard pathologic prognostic features.

Task 4.1: Correlate MRI characteristics to pathologic and proliferative characteristics identified in Specific Aim 1.

- We will correlate MRI characteristics to all pathologic and proliferative characteristics identified above.
- We expect correlation of pathological and radiological analysis to be complete by March 2003.

Task 4.2: Determine if proliferative activity is associated with standard prognostic features alone.

- We will analyze all data and try to determine if proliferative activity is a property of grade and/or size or if it is a biological parameter which might act as a trigger point for progression.

Key Research Accomplishments

Due to the significant delays caused by turnover of key researchers on this study, we have not yet completed the work, but plan to complete manuscripts by May and submit abstracts to ASCO November 2002 and San Antonio June 2003.

Reportable Outcomes

Due to the significant delays caused by turnover of key researchers on this study, there are no reportable outcomes to report at this time.

Conclusions

We anticipate that this project will be completed by April 2003 and have accordingly applied for and received a no-cost extension from UCSF. The database is currently being constructed and will be complete by December 2002. The staining and immunohistochemistry analysis will be complete by February 2003. The MR image and mammography review will be complete by February 2003. We will analyze the data and prepare a final report by May 2003.

References:

1. Ernster VL, Barclay J, Kerlikowske K, Grady D, Henderson C. Incidence of and treatment for ductal carcinoma in situ of the breast. *Journal of the American Medical Association* 1996; 275:913-8.
2. Fisher ER, Constantino J, Fisher B, Palekar AS, Redmond C, Mamounas E. Pathologic findings from the National Surgical Adjuvant Breast Project (NSABP) Protocol B-17. Intraductal carcinoma (ductal carcinoma in situ). The National Surgical Adjuvant Breast and Bowel Project Collaborating Investigators. *Cancer* 1995; 75:1310-9.
3. Lagios MD. Duct carcinoma in situ. Pathology and treatment. *Surg Clin North Am* 1990; 70:853-71.
4. Deng G, Lu Y, Zlotnikov G, Thor AD, Smith HS. Loss of heterozygosity in normal tissue adjacent to breast carcinomas. *Science* 1996; 274:2057-9.
5. Ottesen GL, Graversen HP, Blichert-Toft M, Zedeler L, Anderson JA. Ductal carcinoma in situ of the female breast. Short-term results of a prospective nationwide study. The Danish Breast Cancer Cooperative Group. *Am J Surg Pathol* 1992; 16:1183-96.
6. Habel LA, Daling JR, Newcomb PA. Risk of recurrence after ductal carcinoma in situ of the breast. *Cancer Epidemiol Biomarkers Prev* 1998; 7:689-96.
7. Lagios MD, Margolin FR, Westdahl PR, Rose MR. Mammographically detected duct carcinoma in situ. Frequency of local recurrence following tylectomy and prognostic effect of nuclear grade on local recurrence. *Cancer* 1989; 63:618-24.
8. Silverstein MJ, Poller DN, Barth A. Intraductal breast carcinoma: experiences from the Breast Center in Van Nuys, California. *Recent Results in Cancer Res* 1996; 140:139-53.
9. Silverstein MJ, Barth A, Poller DN. Ten-year results comparing mastectomy to excision and radiation therapy for ductal carcinoma in situ of the breast. *Eur J Cancer* 1995; 9:1425-7.
10. Weidner N. Current pathologic methods for measuring intratumoral microvessel density within breast carcinoma and other solid tumors. *Breast Cancer Res Treat* 1995; 37:169-80.
11. Heimann R, Ferguson D, Powers C, Recant WM, Weichselbaum RR, Hellman S. Angiogenesis as a predictor of long-term survival for patients with node-negative breast cancer. *J Natl Cancer Inst* 1996; 88:1764-69.

12. Gasparini G, Weidner N, Bevilacqua P. Tumor microvessel density, p53 expression, tumor size, and peritumoral lymphatic vessel invasion are relevant prognostic markers in node-negative breast carcinoma. *J Clin Oncol* 1994; 12:454-66.
13. Weidner N. Angiogenesis in cancer. Invited Commentary. *American Journal of Pathology* 1995; 147:9-19.
14. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; 86:353-64.
15. Folkman J. The influence of angiogenesis research on management of patients with breast cancer. *Breast Cancer Res Treat* 1995; 36:109-18.
16. Brown LF, Berse B, Jackman RW, et al. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in breast cancer. *Hum Pathol* 1995; 26:86-91.
17. Kim KJ, Li B, Winer J, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature* 1993; 362:841-4.
18. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995; 1:27-31.
19. Esserman L, Hylton N, George T, Weidner N. Contrast-enhanced magnetic resonance imaging to assess tumor histopathology and angiogenesis in breast carcinoma. *The Breast Journal* 1999; 5:13-21.
20. Buadu LD, Murakami J, Murayama S, et al. Breast lesions: correlation of contrast medium enhancement patterns on MR images with histopathologic findings and tumor angiogenesis. *Radiology* 1996; 200:639-49.
21. Hulka CA, Edmister WB, Smith BL, et al. Dynamic echo-planar imaging of the breast: experience in diagnosing breast carcinoma and correlation with tumor angiogenesis. *Radiology* 1997; 205:837-42.
22. Stillman B. Cell cycle control of DNA replication. *Science* 1996; 274:1659-64.
23. Diffley JF. Once and only once upon a time: specifying and regulating origins of DNA replication in eukaryotic cells. *Genes Dev* 1996; 10:2819-30.
24. Donaldson AD, Blow JJ. The regulation of replication origin activation. *Curr Opin Genet Dev* 1999; 9:62-8.
25. Leatherwood J. Emerging mechanisms of eukaryotic DNA replication initiation. *Curr Opin Cell Biol* 1998; 10:742-8.

26. Tye BK. MCM proteins in DNA replication. *Annu. Rev. Biochem.*; 68:649-686.
27. Stoeber K, Mills AD, Kubota Y, et al. Cdc6 protein causes premature entry into S phase in a mammalian cell-free system. *Embo J* 1998; 17:7219-29.
28. Wu JR, Keezer SM, Gilbert DM. Transformation abrogates an early G1-phase arrest point required for specification of the Chinese hamster DHFR replication origin. *Embo J* 1998; 17:1810-8.
29. Abdurashidova G, Riva S, Biamonti G, Giacca M, Falaschi A. Cell cycle modulation of protein-DNA interactions at a human replication origin. *Embo J* 1998; 17:2961-9.
30. Williams G, Stoeber K. Clinical applications of a novel mammalian cell-free DNA replication system. *Br J Cancer* 1999; 80 Suppl 1:20-4.
31. Williams GH, Stoeber K. A novel immunoenhanced pap test. *Hospital Healthcare Europe* 1999/2000. London: Campden Publishing Limited, 1999.
32. Williams GH, Romanowski P, Morris L, et al. Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. *Proc Natl Acad Sci U S A* 1998; 95:14932-7.
33. Freeman A, Morris LS, Mills AD, et al. Minichromosome maintenance proteins as biological markers of dysplasia and malignancy [In Process Citation]. *Clin Cancer Res* 1999; 5:2121-32.
34. Solin LJ, Kurtz J, Fourquet A. Fifteen-year results of breast-conserving surgery and definitive breast irradiation for treatment of ductal carcinoma in situ of the breast. *J Clin Oncol* 1996; 14:754-63.
35. Solin LJ, Yeh IT, Kurtz J. Ductal carcinoma in situ (intraductal carcinoma) of the breast treated with breast-conserving surgery and definitive irradiation. Correlation of pathologic parameters with outcome of treatment. *Cancer* 1993; 71:2532-42.

APPENDIX

Comments from Principal Investigator in Response to Reviewer

The purpose of this study is to correlate density of contrast enhancement on breast magnetic resonance images (MRI) with pathology characteristics and markers of proliferation and angiogenesis in women with ductal carcinoma in situ (DCIS) of the breast. The specific aims of the study are two-fold. 1) We will first develop a novel method for characterizing DCIS lesions based on cellular proliferative and angiogenic activity within the tumor and surrounding vascular endothelium. Using immunohistochemical techniques, we will perform an extensive pathological review of all cases. This will help us to determine whether this proliferation is found in the DCIS itself, in the surrounding stroma, or in vascular endothelial cells and whether they are proximate. 2) Secondly, we will correlate this angiogenic and proliferative profile with MRI characteristics by performing a tandem radiological review of corresponding MR images. This will allow us to determine whether MR can predict the biological characteristics of DCIS. Thus MR could potentially serve as a surrogate marker of biological behavior. These two aims will lead to a better understanding of the basis and timing for transformation of DCIS which would help us to find more optimal ways to treat DCIS and help us to treat invasive breast cancer and develop strategies for prevention.

Due to the significant delays caused by turnover of key researchers on this study, significant progress was not made on the study until July of 2002. With a new study coordinator working on the study, we have accomplished a number of key research objectives in the past six months. Prior to beginning the pathological and radiological reviews, key investigators on the project convened to discuss relevant criteria and data to be collected in both reviews. After constructing data sheets for both reviews, databases were created to allow for sophisticated data collection and subsequent analysis and a timeline was constructed to ensure that both reviews were conducted in coordination with one another.

Since July 2002, we have completed the initial pathological review of all 90 cases. Based on this initial review of the histology and other characteristic features of DCIS, tissue blocks abundant in DCIS were designated and ordered from our institution's tissue bank. We have already received tissue blocks from ten separate cases and are expect to receive the remaining cases within several weeks. Upon receipt of all the tissue blocks, cutting and staining of the appropriate vascular and proliferative markers will be performed and final pathological analysis will be completed. The angiogenesis stains that will be used include CD34 and CD105, two stains expressed in vessels characteristic of tumors. The proliferative marker used will be Ki67. This stain will serve to elucidate whether tumor, epithelial, or endothelial areas are proliferating. We expect to complete this final pathological review by May 2003.

We have also made significant strides in completing the tandem radiological review of corresponding MRI's. Our new radiologist, Dr. Jessica Leung, has completed review of 10 MR images, including a stratification of the images based on extent, density, and intensity of contrast enhancement of study cases. A number of older MR images initially available only in film format have also been digitized to allow for a more efficient and consistent radiological review. Having successfully applied this stratification system to the first 10 cases, we expect to complete radiological review of the remaining cases by May 2003.

Review of clinical charts has also been completed for all study cases. This has allowed us to glean other information supplementary to our pathological and radiological reviews.

With both the pathological and radiological reviews near completion, we are almost done with the time-consuming data collection phase of the project. Once data collection is complete, we will be able to perform data analysis and determine the correlation between radiological and pathological findings. The radiological and pathological data taken in isolation provide no insights into how they are ultimately correlated, therefore we are unable to present any reportable outcomes at this time. Moving forward, we anticipate none of the staff turnover-related delays that hindered progress during the first two years of the study. We anticipate that this project will be completed by June 2003 and have accordingly applied for and received a no-cost extension from UCSF. We will then compile our findings in a

report to be submitted to your organization in June 2003.